Sutudies on Supplementary Chemotherapy Combined with Sugical Treatment of Carcinoma of the Esophagus

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Studies on Supplementary Chemotherapy Combined with Surgical Treatment of Carcinoma of the Esophagus

by

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Introduction

Recently, surgical treatment of carcinoma of the esophagus has remarkably progressed by the development of pre-, mid- and postoperative managements, However, the five-year survival rate of esophageal cancer has not yet been improved sufficiently. The five-year survival rate in resected cases of esophageal cancer was reported to be 13 per cent by NAKAYAMA, 12.1 per cent by SWEET and 17.3 per cent by GUNNLAUGSSON[9)35)52)]. These results are due to the peculiarity which lymphatic dissemination of esophageal cancer spreads rapidly and widely[1)]. Therefore, for the purpose of improving this rate, surgical treatment of esophageal cancer should be combined with some supplementary therapies. Preoperative radiotherapy of esophageal cancer has been advocated by NAKAYAMA, and the five-year survival rate has been ameliorated considerably[32)33)]. Since a new antitumor antibiotic "Bleomycin (BLM)" was discovered by UMEZAWA, et al. in 1966, a marked effect of this drug has been observed on cancer of the esophagus[2)4)34)] However, the results of surgical treatment and radiotherapy for cancer of the esophagus have not been improved recently. The purpose of this study is to reinvestigate the lymphatic dissemination of esophageal cancer, to investigate fundamentally chemotherapeutic methods combined with surgical treatment for esophageal cancer, and to contribute to the improvement of operative curability.

Chapter I. Investigation on the lymphatic dissemination of esophageal cancer

The lymphatic dissemination of esophageal cancer was investigated in our 47 cases from which squamous cell carcinoma of the esophagus were resected in our clinic from January, 1970 to March, 1974. The following descriptions conform to the descriptive rules of Japanese Society of Esophageal Diseases[17)]. The locations of lesions in our 47 cases are divided as follows : cervical esophagus (Ce) in 8 cases, upper thoracic esophagus (Iu) in 2 cases, middle thoracic esophagus (Im) in 29 cases and lower thoracic esophagus (Ei) in 8 cases.

1. Relationship between the depth of invasion and lymph node metastases or intramural metastases of esophageal cancer (Table 1)

In 18 cases, cancer did not penetrate the muscularis propria of esophageal wall, while, in 29 cases, it invaded the adventitia in various degrees. In cancer of the middle thoracic esophagus, occurrence of lymph node metastases was rather more frequent in the former

Key words : Band culture method, Bleomycin, Chemotherapy, Cu-BLM, Esophageal surgery.
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Table 1 Relationship between the depth of invasion and lymph node metastases or intramural metastases of esophageal cancer

Location of lesion	No. of cases	Depth of invasion	Radiation		No radiation		Direction of intraepithelial invasion
			n_1～n_4 metastases		n_1～n_4 metastases		
Ce	8	mp	1 3		—		—
		a_1～a_3	2 3		1 2		—
Iu	2	mp	—		—		—
		a_1～a_3	2 2		—		—
Im	**29**	mp	6/9	**66%**	4/5	**80%**	upward 2
		a_1～a_3	3/7	**43%**	5/8	**62%**	downward 1
Ei	8	mp	0 1		—		downward 1
		a_1～a_3	2 3		3 4		

Ce cervical esophagus Iu : upper thoracic esophagus
Im : middle thoracic esophagus Ei . lower thoracic esophagus
mp : muscularis propria a adventitia

group, regardless of preoperative radiotherapy was combined or not. This fact shows that the spread by way of lymphatics becomes more frequent when invasion of cancer infiltrates into the submucosal layer because of its mode of lymphatic drainage. In three cases out of 29 cases in the middle thoracic group, intraepithelial invasion from the main focus of cancer was seen in one direction only. In two cases it was upward, whereas in another two cases it was downward. These directions of invasion completely corresponded to the direction of intramural lymphatic flow.

2. Relationship between lymph node metastases and annular cancer infiltration or preoperative radiotherapy (Table 2).

Table 2 Relationship between lymph node metastases and annular cancer infiltration or preoperative radiotherapy

Location of lesion	No. of cases	n_1～n_4 metastases		n_3～n_4 metastases	
		Radiation	No radiation	Radiation	No radiation
Ce	8	3 6	1 2	1 6	1 2
Iu	2	2 2	—	0 2	—
Im	**29**	9 16	9 13	7 16	2 13
	incomplete annular	3 6	6 8	3 6	1 8
	complete annular	6 10	3 5	4 10	1 5
Ei	8	2 4	3 4	2 4	3 4

In cases who had undergone the preoperative betatron therapy at the dose of 3000 or 5000 rads, lymph node metastases, especially remote metastases to the third and the fourth groups of lymph nodes occurred more frequently as compared with the group who had not received radiotherapy. In the middle one-third group, frequent occurrence of lymph node

metastases in the neck and in the upper abdomen was observed. This tendency was more pronounced when the annular infiltration of lesion into the esophageal wall became complete.

Chapter II. Fundamental investigation on chemotherapy for esophageal cancer by measuring anticancer drug levels in the esophagus, other organs and body fluids.

Concentration of anticancer drug in the various body fluids or organs is measured by bioassay. This method uses the antibacterial activity of anticancer drug against microorganism. Correlation between logarithm of drug concentration (log C) and length of inhibition zone (l) is represented as follows;

$l = \alpha \log C + \beta$ (α or β : constant)

Standard curve is made by the length of inhibition zone corresponding to the known concentration of anticancer drug. Drug level in the specimen is estimated by a value on the concentration axis corresponding to the length of inhibition zone induced by unknown levels in the specimen.[5)24)] In practice, the "Band culture method" in bioassay which was invented by OKUBO in 1955, was used in this experiment[40)]. Various conditions of measurement were determind by the preliminary investigations, as shown in Table 3.

Table 3 Various conditions of measurement of anticancer agent concentration

Specimen	1. Sterile organs are resected 2. Saline solution or PBS is added 3. Organs are homogenized and extracted at 0C° for 2 to 48 hours		
Method of measurement	Band culture method		
Anticancer agents	BLM	5-FU	MMC
Strain of test organisms	*B subtilis* PCI 219	*St. aureus* 209-P	*E. coli* B
Number of organisms	2.1×10^7/cc	6.5×10^7/cc	1.0×10^7/cc
Medium	M.-H. medium	M.-H. medium	Nutrient agar
Incubation time at 37°C (hrs.)	5 to 7	7 to 9	5 to 7
Minimum Inhibition Concentration (mcg/cc)	0.25	0.025	0.0025

Composition of each medium

Mueller-Hinton medium		Nutrient agar	
meat extract	300g	peptone	5g
casamino acid	17.5g	meat extract	3g
starch	1.5g	agar	15g
pH	7.4±	Aq. dest.	1l
		pH	6.2~6.8

1. Blood and thoracic duct lymph levels of Bleomycin in dogs following intravenous administration

Materials and methods

Adult mongrel dogs weighing about 9 kg were used. The dogs were kept fasting for 12 hours prior to surgery and anesthetized by intramuscular injection of Ketamine chloride at the dose of 10 mg/kg. During surgery airway was provided by an endotracheal intubation and

oxygenated by AIKA's pressure preset respirator with the room air under positive pressure of 20 cm H_2O and with controlled respiration of 20 times per minute. Through a paramedian incision in the left cervical region, the thoracic duct was dissected and ligated just proximal to the jugular-subclavian junction. An elastic polyethylene tube, No. 4 in size, which was heparinized, was cannulated into the dilated proximal portion of the thoracic duct. BLM solution was injected intravenously into dogs at the dose of 0.5mg/kg. Blood samples were taken from the femoral vein of dogs from 5 to 30 minutes after injection. Simultaneously, thoracic duct lymph samples were collected in glass tubes every 5 minutes for 30 minutes after injection.

Results :

Peak level of concentration appeared a little later in the thoracic duct lymph as compared with that in the peripheral blood. Blood level of BLM decreased from 5 minutes after injection, while thoracic duct lymph level increased gradually throughout the experiment (Fig. 1).

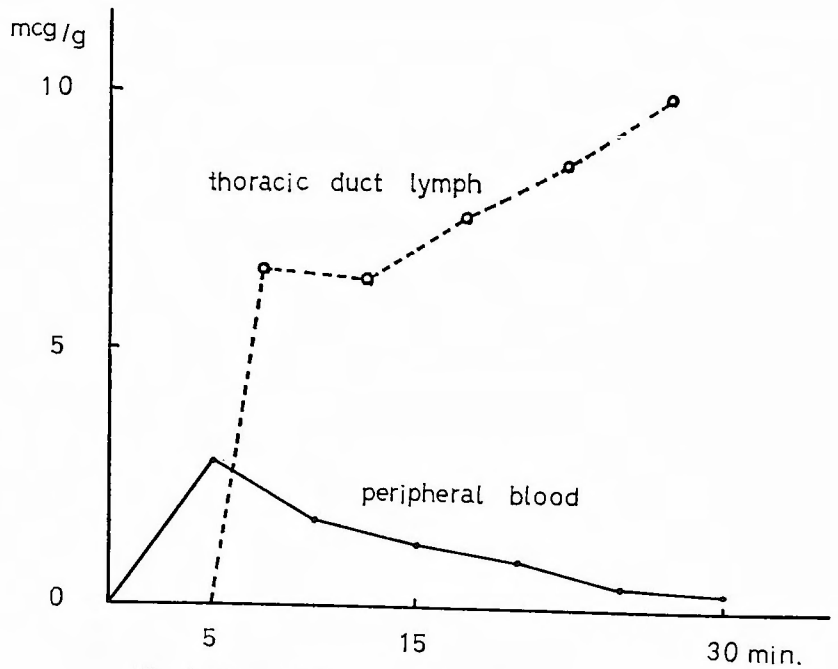

Fig. 1 Blood and thoracic duct lymph levels of Bleomycin in dogs following intravenous administration

2. Comparison of blood levels of Bleomycin following 7.5 or 15 mg injection into cancer patients.

Materials and methods

Blood levels of BLM in 8 cases of esophageal cancer were measured. In 5 cases, 15 mg of BLM, and in 3 cases 7.5mg were injected intravenously. A woman (66y., 50.8kg) in this group was injected 7.5 and 15 mg, at different times.

Results

Blood levels of BLM in cancer patients arrived at a peak level 5 min. after injection and

decreased gradually thereafter. Blood levels did not have a close relationship to the administered dosage (Fig. 2-A). However, the difference in blood levels between 7.5 mg injected and 15 mg injected groups was found, when the mean levels in these groups were compared with each other. Blood levels in one case, who received each one of these doses at different times, showed the same tendency (Fig. 2-B).

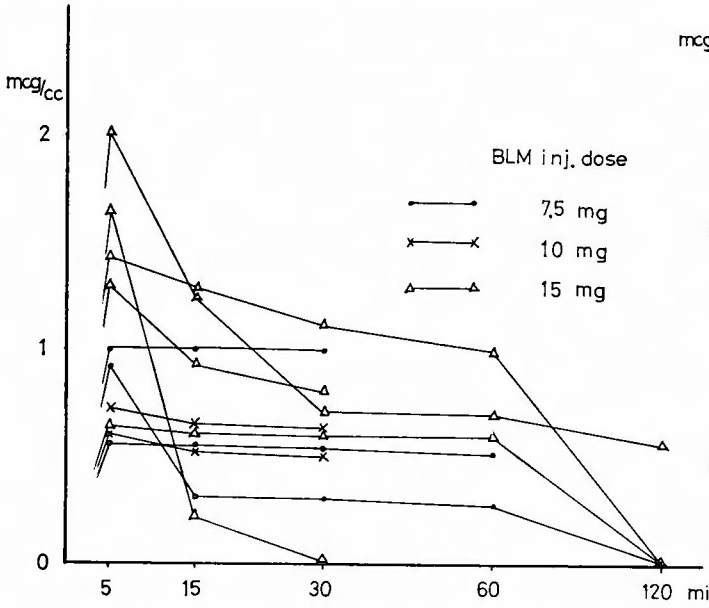

Fig. 2-A Blood levels of Bleomycin in clinical cases following intravenous administration

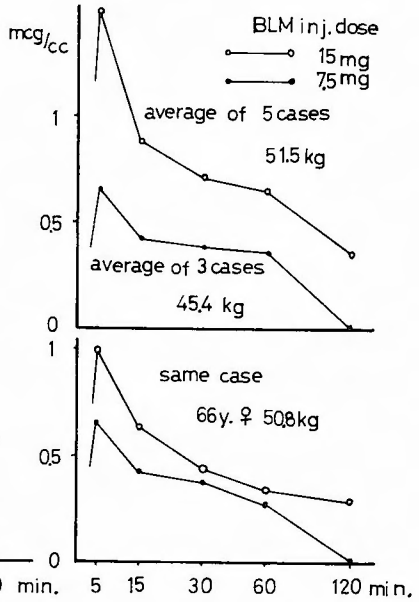

Fig. 2-B Comparison of blood levels of Bleomycin following 7.5mg or 15 mg injection in cancer patients

3. Bleomycin concentration in various organs of mice and dogs

Experimental animals and methods

Five mice of dd-strain were intravenously administered with 33.3 mg of BLM or 100 mg of copper chelating BLM (Cu-BLM). Mongrel dogs were intravenously injected with 0.5 or 1mg/kg of BLM. The animals were sacrificed by intravenous injection of Thiopental 30 min. after BLM injection. The removed tissues were minced with scissors and homogenized in a waring blender with 2 to 4 times the volume of physiologic saline or pH 7.2 phosphate buffer solution. These homogenates were kept in a refrigerator at 4°C for 48 hours. The resultant supernatant fluids were used for the measurement of anticancer drug concentrations in various tissues. Then, BLM concentrations in homogenates of various organs were assayed.

Results

Table 4 shows tissue levels of BLM in mice and dogs following the injection. BLM level in the esophagus showed the highest value among various organs. Relatively high levels of BLM were detected in the lung, kidney, spleen and liver. BLM in the skin of mice was at a high level, but in dogs BLM was not observed.

4. Distribution of various anticancer drugs in each layer of the esophagus following intravenous injection

Table 4 Bleomycin concentrations in various organs of mice and dogs (mcg/g)

Experimental animals	Mouse			Dog		
Agents	Cu-BLM 100mg/kg i.m.			BLM 33.3mg/kg i.m.	BLM 0.5 mg/kg i.v.	BLM 1mg/kg i.v.
Interval after injection(min.)	30	60	120	30	30	30
Esophagus	92.5	28.0	2.84	10.5	4.17	2.54
Kidney	73.0	46.4	7.0	12.7	3.96	2.4
Lung	62.0	26.8	2.4	7.6	2.4	0
Liver	18.75	22.8	2.0	0	2.9	1.3
Spleen	16.2	4.48	2.0	0	3.2	1.44
Stomach	—	18.2	4.2	—	0	0
Intestine	—	2.68	2.1	—	0	0
Lymph node	—	—	—	—	2.65	1.28
Skin	—	60.0	5.0	17.75	0	0

Materials and methods

BLM, 5-Fluorouracil (5-FU) and Mitomycin C (MMC) were given by intravenous injection into dogs and operative cases of esophageal cancer. The tissue of esophagus removed after injection was divided into the mucous membrane and the muscular layer, and the drug concentration in homogenate of each layer was assayed immediately.

Results

BLM distribution was detected only in the mucous membrane of the esophagus. The distribution of 5-FU was detected in a small amount in the muscular layer of dog's esophagus, but not detected in both the mucous membrane of dogs and human esophagus of operative cases. The distribution of MMC was not detected at all in the esophagus of dogs and cancer patients (Table 5).

Table 5 BLM concentrations in each layer of the esophagus

30 minl after i.v. administration

Anticancer agents	BLM		5-FU		MMC	
Species	dog	cancer pt.	dog	cancer pt.	dog	cancer pt.
Body weight (kg)	11	29.5	9	36.4	7	45.6
Dose (mg/kg)	1.0	0.18	5.0	7.0	0.5	0.04
Mucous membrane (mcg/g)	2.54	1.8	0	0	0	0
Muscular layer (mcg/g)	0	0	0.37	0	0	0

5. Bleomycin levels in various organs following various types of administration

The above-mentioned experimental results showed that BLM distributed in the esophagus and regional lymph nodes on high levels. Various types of drug administrations were investigated in order to prevent the lung from complication, which is frequently caused by BLM administration, and to increase the drug level in the esophageal lesion. The author tried to find a new method to administer anticancer agents. Such methods of administration,

as intraarterial injection into the ascending branch of the left gastric artery, local injection into the wall of the esophagus and administration into the lumen of the esophagus, were investigated. BLM levels in these administration were compared with those in the systemic administration by intravenous injection.

Materials and methods

Mongrel dogs weighing about 10 kg were anesthetized by the above-mentioned method. In one group of dogs, laparotomy was performed through upper median incision. Thereafter, the ascending branch of the left gastric artery was dissected and other branches were ligated. A polyethylene tube of No. 4 in size was cannulated into the ascending branch, and BLM solution of 0.5 mg per kilogram in body weight was injected into the catheter by intra-arterial one-shot injection. In another group of dogs, right thoracotomy was performed through the fifth or sixth intercostal space. BLM solution of 0.5 mg/kg was injected into the submucosal layer of the lower esophagus. Furthermore, in some dogs the thoracic duct drainage was combined with these methods. Various organs were removed 30 minutes after BLM injection. Tissue homogenates were prepared by the above-mentioned method.

Results

Concentration of BLM in the esophagus and the intrathoracic regional lymph nodes increased following local injection into the esophageal wall, intravenous injection and selective intra-arterial injection into the ascending branch of the left gastric artery, in the order mentioned. BLM levels in the lung was lowered following intravenous injection, selective intraarterial injection and local injection, in the order mentioned. BLM levels in the esophagus and the regional lymph nodes following intravenous injection were higher than those following intravenous injection combined with lymphatic drainage (Table 6).

Table 6 Bleomycin levels in the esophagus, regional lymph node and lung following various types of administration (mcg/g)

30 min. after injection of 0.5mg/kg into dogs

	Esophagus	Regional lymph node	Lung
Local injection with thoracic duct drainage	115.22	2.84	1.64
Intravenous injection	20.88	2.68	2.48
Intravenous injection with thoracic duct drainage	13.88	2.16	2.59
Selective intraarterial injection with thoracic duct drainage	10.03	1.76	1.84

6. Bleomycin levels in each portion of the esophagus 30 minutes after the administration in dogs with thoracic duct drainage

Materials and methods

BLM of 0.5mg/kg were administered by intravenous injection, selective intraarterial injection and local injection into the lower thoracic portion of the esophagus in dogs with thoracic duct drainage. According to the descriptive rules of Japanese Society of Esophageal Diseases, each portion of the esophagus was removed separately. BLM levels in each portion were determined.

Results

BLM level by local injection into the lower thoracic esophagus showed the highest value at the site of injection. In selective intraarterial injection, BLM level in the lower thoracic esophagus was higher than that in the upper thoracic esophagus. In intravenous injection, BLM concentrations in each portion of the esophagus showed almost the same level (Table 7).

Table 7 Bleomycin levels in each portion of the esophagus 30 min. after the administration in dogs with thoracic duct drainage (mcg/g)

0.5 mg/kg of BLM inj.			
	Intravenous injection	Selective intraarterial injection	Local injection
Ce	2.64	1.53	2.24
Iu	2.48	2.04	2.28
Im	3.2	2.24	8.4
Ei	2.84	2.24	100.0
Ea	2.72	1.98	2.3

Ce : cervical esophagus Iu : upper thoracic esophagus
Im : middle thoracic esophagus Ei lower thoracic esophagus
Ea : abdominal esophagus

7. Bleomycin levels in each portion of the esophagus 30 minutes after the intramural injection in dogs with thoracic duct drainage

Materials and methods

Right thoracotomy was carried out through the 6th intercostal space in dogs weighing about 7 kg. BLM solution of 1 mg/kg was injected into the wall of the lower thoracic esophagus. In other dogs, intramural lymphatic flows of the esophagus were interrupted by ligating the transitional portions to the middle thoracic and abdominal esophagus. Thereafter, BLM of the same dosage was injected into the wall of the lower thoracic esophagus. BLM levels in each portion of the esophagus in both groups were assayed 30 minutes after local injection.

Results

High levels of drug were found in the injected portions. In the control group, BLM distributed as far as the middle thoracic and abdominal esophagus at the levels with gradual decrease. In the blockage group, most of the BLM remained in the injected portion, but BLM was scantily found in the oral and aboral portions (Fig. 3).

8. Influence of changes in lymphatic or blood flow in the esophagus on tissue levels of Bleomycin following intramural injection

The peculiarity of lymphatic flow in the esophagus was shown in the above-mentioned results. The influence of changes in lymphatic and blood flow in the esophagus on its BLM levels following local injection was investigated in this experiment.

Materials and methods

1) The thoracic duct drainage was done in dogs. 2) The right lymphatic and thoracic ducts were ligated. 3) The azygos vein and the first to the twelfth intercostal veins on the right side were ligated after thoracotomy. BLM of 1 mg/kg was injected into the middle thoracic esophagus after these procedures. In another group of dogs, local injection was performed into the middle thoracic esophagus without these procedures. BLM levels 30 minutes after

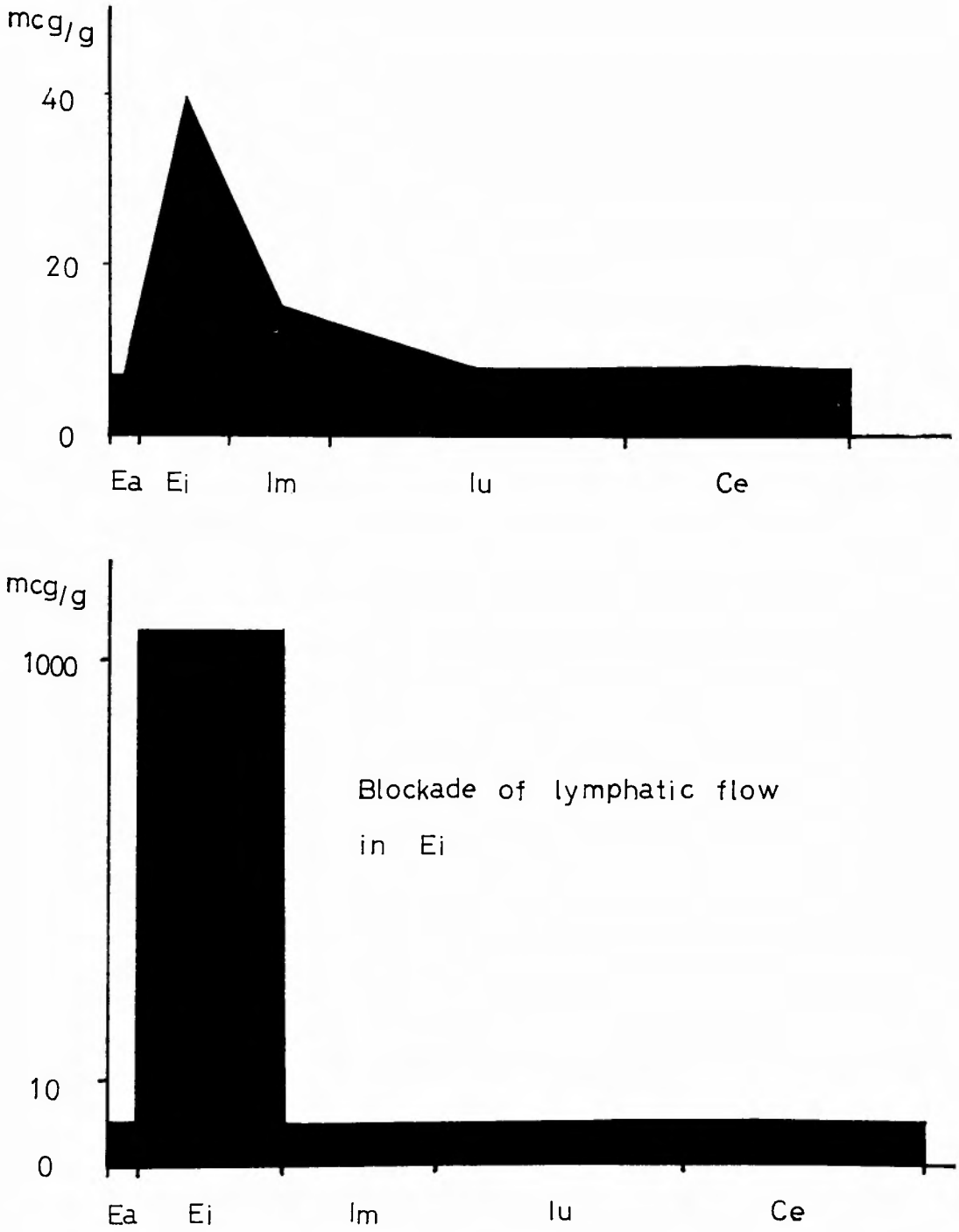

Fig. 3 Bleomycin levels in each portion of the esophagus 30 minutes after the intramural injection in dogs with thoracic duct drainage

injection were assayed and compared with each other.

Results

BLM levels in the esophagus in dogs with thoracic duct drainage was lower than those without drainage because lymphatic flow was accelerated by the drainage of esophageal lymphatics. On the contrary, BLM levels in the esophagus in dogs with ligation of the right lymphatic and thoracic ducts was higher than those without the procedure because of the stagnation of lymph. Ligation of the azygos and intercostal veins gave more higher levels of drug. It may be attributable to the prevention of venous return from the esophagus by these procedures (Table 8).

Table 8 Influence of changes in lymphatic or blood flow in the esophagus on tissue levels of Bleomycin following intramural injection (mcg/g)

Ligation of azygos and intercostal veins	24.2
Ligation of thoracic and right lymphatic ducts	18.1
Intramural injection into mid-thoracic esophagus	10.52
Intramural injection into mid-thoracic esophagus with thoracic duct drainage	10.02

30 min. after the local injection of BLM, 1mg/kg, into the mid-thoracic esophagus

9. Influence of interruption of lymphatic flow in the esophagus on Bleomycin levels in regional lymph nodes

It was clarified statistically in Chapter I that remote lymphatic dissemination frequently occurred when annular infiltration of cancer lesion into the esophageal wall became complete. The phenomenon of lymphatic dissemination will be investigated in this experiment.

Materials and methods

Right thoracotomy was carried out through the 5th intercostal space in dogs. Continuous No. 4 silk suture through all layers of the whole circumference of the esophagus was performed at each level. When lymphatic flow in the esophagus was blocked by suture and scar-formation 2 weeks after the procedure, BLM solution of 1mg/kg was injected into the esophagus aboral to the blockade by rethoracotomy. BLM concentrations in the cervical, intrathoracic and intraperitoneal regional lymph nodes were measured 30 minutes after the injection. As a control, BLM were injected into each portion without interruption of the esophageal wall. BLM levels in this group were assayed similarly. Drug levels in these lymph nodes in both groups were compared with each other.

Results

BLM distribution in remote lymph nodes, such as lateral cervical and mesenteric nodes (Nos.100, 14), etc., increased frequently after every blockade at the upper, middle and lower thoracic esophagus. BLM distribution in intrathoracic regional lymph nodes, such as upper thoracic paraesophageal and bifurcation nodes (Nos. 105, 107), etc., decreased after blockade at the lower thoracic esophagus. However, after blockade at the upper or mid-thoracic portion, distribution of the drug in these lymph nodes increased (Fig. 4).

10. Bleomycin concentrations in regional lymph nodes, lung and esophagus following local injection into the submucosa of the cervical esophageal fistula

Postoperative recurrence of cervical and thoracic esophageal cancer originated most

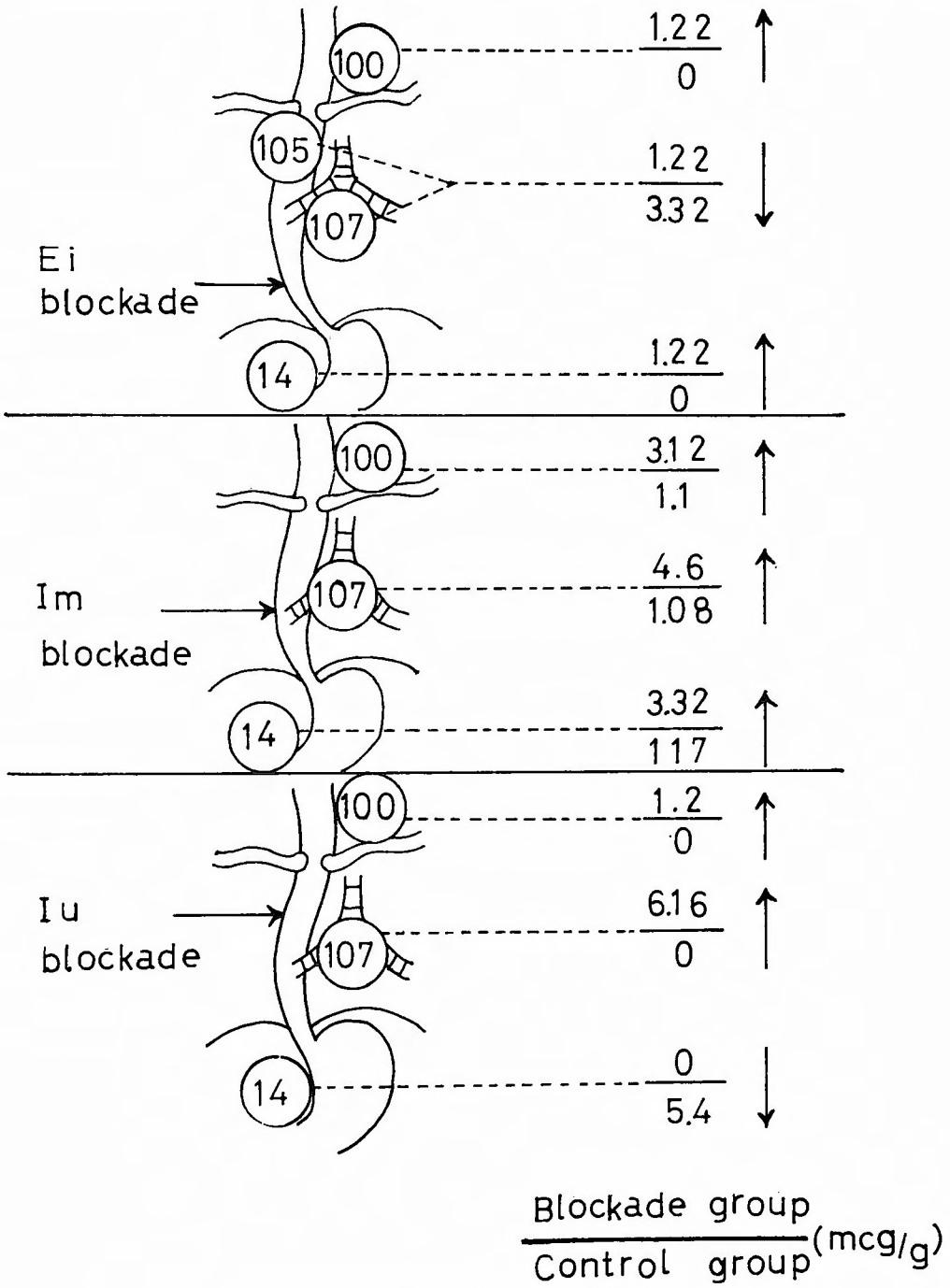

Fig. 4 Influence of interruption of lymphatic flow in the esophagus on Bleomycin levels in regional lymph nodes

likely in residual metastases in the cervical and upper mediastinal lymph nodes. To cope with these metastases, the next experiment was undertaken.

Materials and methods

Cervical esophageal fistula was constructed in the left cervical region following transection of the upper thoracic or cervical esophagus in dogs. BLM of 1 mg/kg was injected into the submucosa of the esophageal fistula. Drug levels in lymph nodes and lung were determined 30 minutes after local injection.

Results

The distribution of BLM was found in the cervical lymph nodes on a high level, and in the thoracic lymph nodes, such as upper paraesophageal and bifurcation nodes (Nos. 105, 107), etc. in a small amount. However, the distribution in the lung was not detected (Table 9).

Table 9 Bleomycin levels in regional lymph nodes, lung and esophagus following local injection into the submucosa of cervical esophageal fistula (mcg/g)

Tissues	Site of esophageal transection	
	Upper thoracic	Cervical
Cervical regional lymph nodes	10.56	2.2
Intrathoracic regional lymph nodes	0.63	2.84
Intraperitoneal regional lymph nodes	—	0
Lung	0	0
Esophagus	60.57	24.09

11. Bleomycin administration into the lumen of the esophagus

The administration of antitumor drug into the lumen of the alimentary tract was first carried out in clinical cases of colo-rectal cancer[3)43)]. NISHIMURA used this type of administration for the unresectable cases of esophageal cancer[37)]. However, fundamental experiment on this type of administration has not yet been performed.

Materials and methods

The uppermost and lowermost portions of the thoracic esophagus were ligated with silk threads and closed in dogs and cancer patients. BLM solution was injected into the blied lumen of the esophagus. Following the injection, various organs, such as the esophagus, regional lymph nodes, etc., were resected. BLM levels in organs were assayed. The levels in peripheral blood and thoracic duct lymph in dogs were measured during 6 hours following the injection. The esophagus was washed out with physiologic saline solution immediately after its removal.

Results

BLM was absorbed into body fluids rapidly from esophageal mucosa. Following BLM administration of 60 mg in 20 cc of physiologic saline solution, its levels were detected in peripheral blood and thoracic duct lymph. Furthermore, its levels in the thoracic duct lymph were higher than that in peripheral blood for 6 hours after the injection. Its distribution was found also in the esophagus and other organs (Fig. 5, Table 10). In each case of the upper

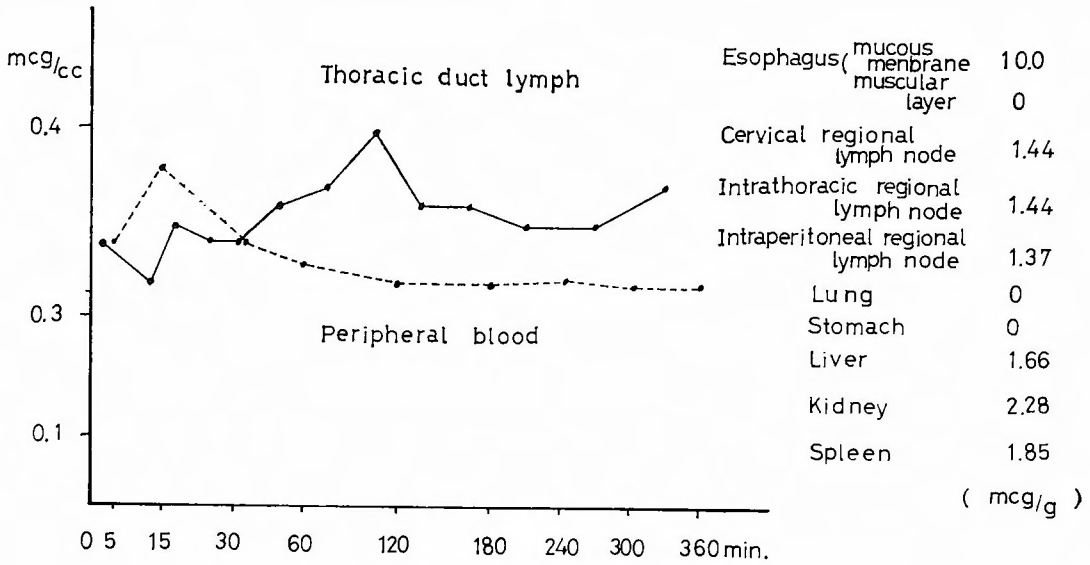

Fig. 5 Blood, lymph and tissue levels of Bleomycin following its administration into the lumen of the esophagus

Table 10 Tissue levels of Bleomycin following its administration into the lumen of the esophagus (mcg/g)

Species		Dog 6kg	Dog 5kg	Im ♂ 39kg	Ei ♂ 49kg
Duration of administration (min.)		60	120	30	150
Esophagus	cancer lesion	—	—	1.06	1.65
	mucous membrane	6.16	3.64	10.0	1.62
	muscular layer	0	0	9.45	0
Regional lymph nodes	cervical	1.37	0	—	—
	intrathoracic	3.32	1.06	1.08(108/109)	1.68(110)
	intraperitoneal	1.56	—	—	1.59(3,7,9)
Lung		1.74	1.15	—	—
Liver		1.74	1.21	—	—
Spleen		4.56	1.36	—	—
Kidney		6.48	1.36	—	—

and lower thoracic esophageal cancer, 15 mg of BLM in 20 cc of physiologic saline solution was administered into the blind lumen of the esophagus which was closed by ligating at the sites oral and aboral to the lesion. BLM levels were detected in resected tissue specimen on the high levels 30 and 150 minutes after its administration (Table 10).

12. Residual time of Bleomycin in various organs

Materials and methods

BLM at 3 different doses of 0.5, 1 and 2 mg per kilogram were intramuscularly injected into 3 groups of dogs, respectively, 3 times every other day. BLM concentration was

determined 25 to 27 hours after its injection. Fifteen mg of BLM was also administered into the cervical esophagus. BLM levels were assayed in the esophagus and other organs 7 days after local injection.

Results

Residual doses of BLM in various organs showed almost the same value irrespective of the interval after the injection. A slight dose of BLM remained in the esophagus even 25 to 27 hours after the intramuscular injection, and 7 days after the intramural injection into the cervical esophagus. Especially, following the intramuscular or local injection, BLM was detected only in the mucous membrane of the esophagus, but not in the muscular layer of the esophagus and all layers of the stomach (Table 11).

Table 11 Tissue levels of Bleomycin one to seven days after the intravenous and local injection

(mcg/g)

Dose and type of administration (mg/kg)		0.5 i.m.	1.0 i.m.	2.0 i.m.	1.8 intramural inj. into Ce
Interval after injection		25 hrs.	26 hrs.	27 hrs.	7 days
Esophagus	mucous membrane	2.76	2.76	2.88	1.3
	muscular layer	0	0	0	0
Stomach	mucous membrane	0	0	0	0
	muscular layer	0	0	0	0
Lymph node		—	0	2.48	1.2
Lung		0	2.3	0	1.2
Liver		2.6	2.52	2.44	1.28
Spleen		2.6	2.48	3.32	1.32
Kidney		3.4	4.24	2.88	1.74

13. Bleomycin levels in the esophagus and lymph nodes in cancer patients

Materials and methods

BLM was injected into 14 preoperative cases of esophageal cancer at a dose of 7.5,10 and 15 mg. The drug levels in the mucous membrane and muscular layer of adjacent normal esophagus, cancer lesion and regional lymph nodes were assayed 25 to 150 minutes following intravenous injection (Table 12).

1) BLM levels in the esophagus in cancer patients and comparison between those levels in normal esophagus and cancer lesion

Results

In all cases, BLM levels in esophageal cancer lesions showed a higher level than those in the adjacent normal esophagus. This is thought to be due to the affinity of the drug for the tissue of squamous cell carcinoma. Moreover, BLM was found only in the epithelial layer, and not in the muscular layer of the human esophagus. BLM was detected in adenocarcinoma lesion, when it was administered into esophago-cardial cancer patients (Fig. 6, Table 12).

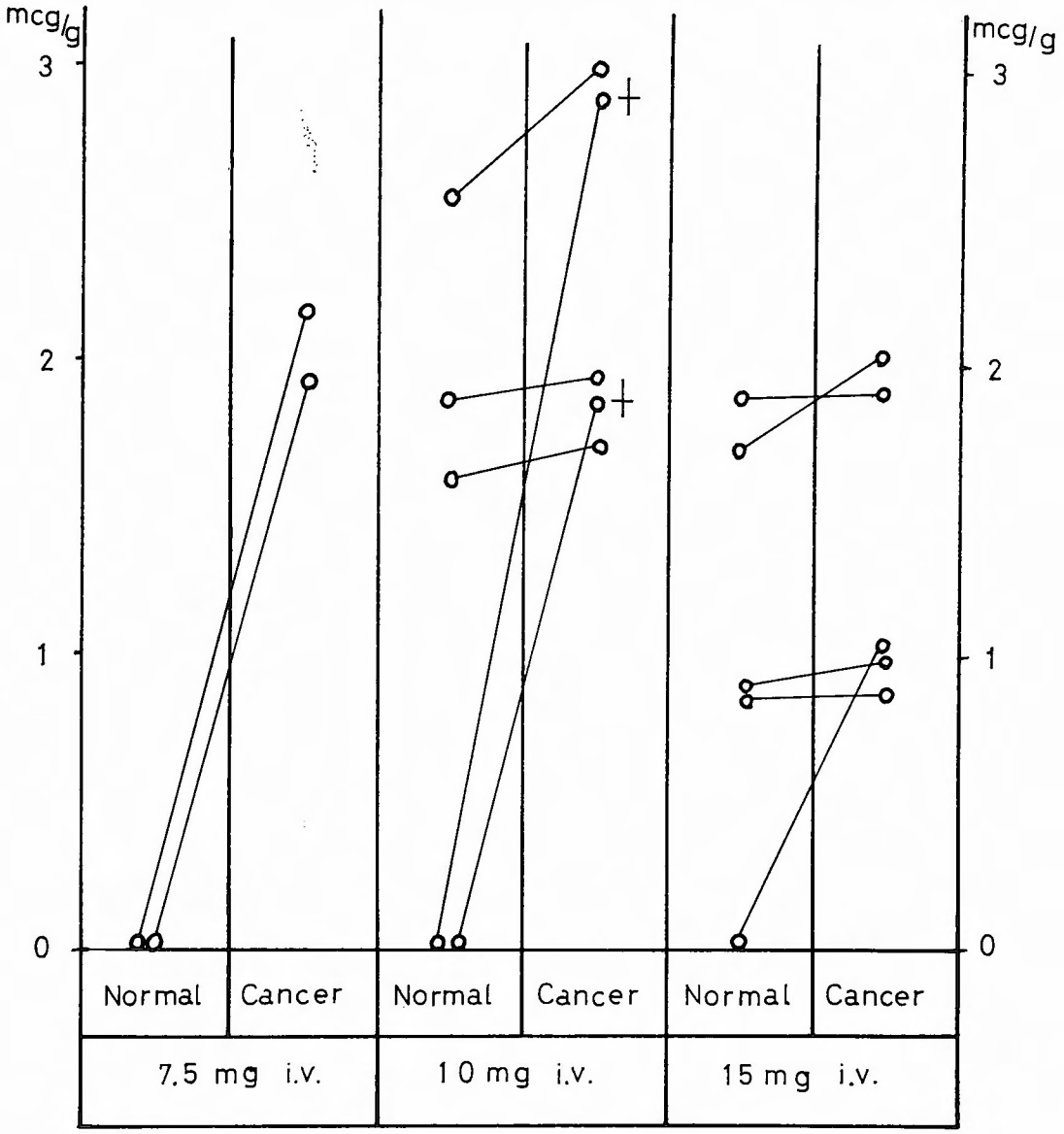

Fig. 6 Bleomycin levels in the esophagus in cancer patients and comparison between those levels in the normal esophagus and cancer lesion (+ : adenocarcinoma)

2) Influence of preoperative radiotherapy on BLM levels in the esophagus of cancer patients

Results

BLM levels were determined in twelve cases of squamous cell carcinoma of the esophagus. Five cases were irradiated by betatron of 2700 to 3600 rads preoperatively, and seven cases had not received the preoperative betatron therapy. The drug levels in the adjacent normal esophagus decreased after the preoperative radiotherapy (Fig. 7).

Table 12 Bleomycin levels in the esophagus and lymph nodes in cancer patients

(mcg/g)

Cases No.	age	sex	Preoperative betatron therapy (rads)	Inj. dose of BLM (mg) i.v.	Cancer lesion	Adjacent normal esophagus: mucous membrane	Adjacent normal esophagus: muscular layer	Lymph nodes	Histological types
1	55	♂	2700	7.5	2.16	0	0	3.18	moderately different.
2	58	♀	3300		1.92	0	0	—	well different.
3	59	♂	—		3.0	2.55	0	3.0	poorly different.
4	65	♀	—		1.72	1.59	0	—	well different.
5	69	♀	—	10	1.93	1.86	0	—	well different.
6	50	♀	—		1.89	0	0	—	adenocarcinoma
7	72	♀	—		2.88	0	0	—	adenocarcinoma
8	64	♂	3600		—	2.2		—	poorly different.
9	68	♂	3000		2.01	1.7		1.71	well different.
10	69	♂	3000		1.1	1.68		1.1	moderately different.
11	61	♂	—	15	—	1.8	0	—	well different.
12	61	♂	—		1.89	0	0	1.78	well different.
13	45	♂	—		1.05	0.88	0	0.96	well different.
14	69	♀	—		0.84	0.8	0	—	well different.

3) BLM levels in regional lymph nodes of the esophagus

Results

BLM concentrations in the regional lymph nodes of thoracic esophageal cancer showed almost the same values as those in cancer lesion on the whole, and were higher than those in the adjacent normal esophagus (Table 12).

14. 5-Fluorouracil and Mitomycin C levels in the stomach of the cancer patients

BLM does not always have an effect on the adenocarcinoma in the esophagocardial leison which originated in the upper portion of the stomach and invaded the lower esophagus. Five-FU and MMC were administered into dogs and operative cases of gastric cancer[47]. The drug levels in cancer lesion, mucous membrane and muscular layer of the stomach and regional lymph nodes were measured by the same method.

Results

Five-FU levels were detected in all layers of the stomach. Moreover, in adenocarcinoma lesion and regional lymph nodes 5-FU levels were assayed on the high levels. The levels in the tissues of stomach decreased gradually in the course of time. MMC levels were not detected in the stomach of dogs and human being (Table 13).

Short summary

Effect of 5-FU against adenocarcinoma has had used widely[59]. Five-FU distributed in

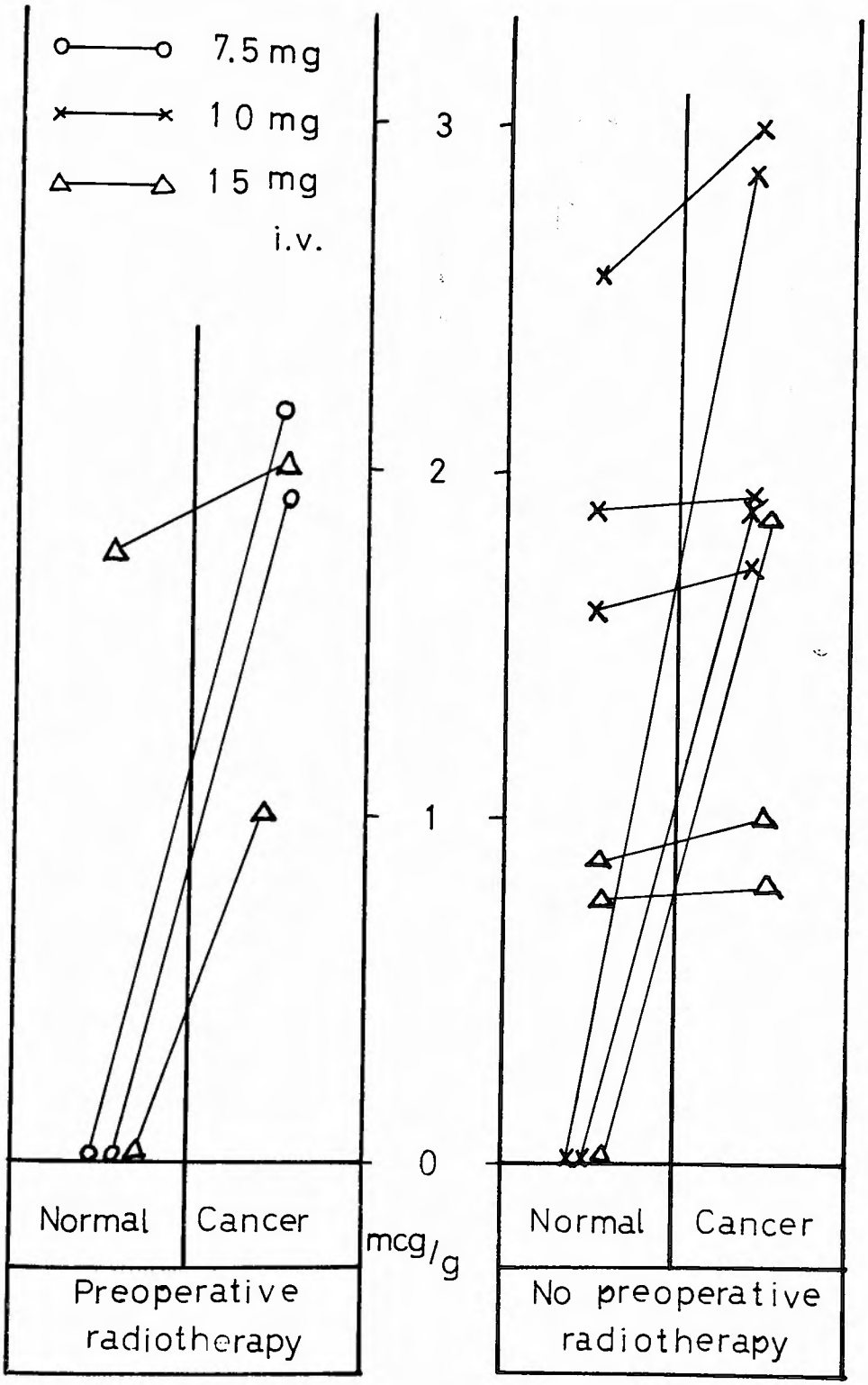

Fig. 7 Bleomycin levels in the esophagus of cancer patients who had undergone preoperative radiotherapy or not

Table 13 Tissue levels of 5-FU in various organs of gastric cancer patients
500 mg of 5-FU i.v. injection (mcg/g)

Cases			73y., ♀, 38kg	56y., ♂, 46kg	71y., ♂, 38kg
Interval after injection (min.)			13	30	99
Tissues	Adenocarcinoma lesion		16.8	—	4.5
	Stomach	mucous membrane	36.8	1.69	0.19
		muscular layer	19.8	0.64	0
	Regional lymph node		15.6	—	0.25

cancer lesions at a high level. This results may indicate its effect on adenocarcinoma. However, the effect of MMC has not yet been determined because its distribution has not yet been found in the stomach.

Chapter III. In vitro inactivation of Bleomycin by the esophagus, stomach and various tissues of carcinoma

Effect of anticancer drugs on cancer lesion was influenced by their concentration and duration in the lesion. Taking into consideration this mode of action of anticancer drug, inactivation of BLM in cancer tissue and other normal tissue was investigated in this experiment. The activity of antitumor antibiotic decreases under the influence of tissue and body fluids[22]. The inactivation of antitumor antibiotic by tissue homogenates is ascribed to the adsorption at 0°C and the enzymatic decomposition at 37°C.

Materials and methods

Various tissues of esophageal and gastric cancer patients and dogs were resected operatively. The fresh tissues, such as normal esophagus, stomach and squamous cell carcinoma, adenocarcinoma lesion etc., were placed into physiologic saline solution, homogenized and made into 30% emulsions. These tissue homogenates were mixed with equal volumes of BLM solution in vitro, and drug inactivation was investigated at 0°C and 37°C, respectively. Drug levels in the mixtures were measured by the band culture method every 15 minutes for two hours after the mixing.

1. Inactivation of Bleomycin by tissues of the esophagus and stomach

Results

Inactivation of BLM by tissue homogenates was observed at 0°C and 37°C. This inactivation occurred more intensely at 37°C than at 0°C. It was seen that BLM was inactivated by the adsorption and the enzymatic decomposition of these tissues. However, the rate of inactivation decreased gradually in the course of time. Inactivation of BLM caused by the gastric tissue was higher than those caused by the esophageal tissue. The mucous membrane and muscular layer of normal esophagus and stomach showed almost the same degree of BLM inactivation (Table 14).

2. Inactivation of Bleomycin by tissues of squamous cell carcinoma and adenocarcinoma

Results

Inactivation of BLM caused by tissue of squamous cell carcinoma occurred slightly, but those caused by tissue of adenocarcinoma occurred intensely (Fig. 8).

Table 14 Inactivation of Bleomycin by normal esophageal or gastric tissues in dogs (mcg/cc)

BLM 20 mcg/cc, 30% Homogenate

Temperature	Homogenized tissue		Reaction time (min.)				
			0	15	30	60	120
37°C	Esophagus	mucous membrane	4.95	4.1	3.55	1.55	1.14
		muscular layer	7.95	6.62	6.62	2.88	0.48
	Stomach	mucous membrane	9.5	6.62	3.12	0.86	0.86
		muscular layer	12.6	11.4	6.9	5.25	1.55
0°C	Esophagus	mucous membrane	16.8	14.4	6.9	4.8	1.6
		muscular layer	18.8	9.5	8.8	5.25	2.88
	Stomach	mucous membrane	25.0	14.4	7.4	4.95	1.14
		muscular layer	11.2	10.4	10.4	5.25	2.6

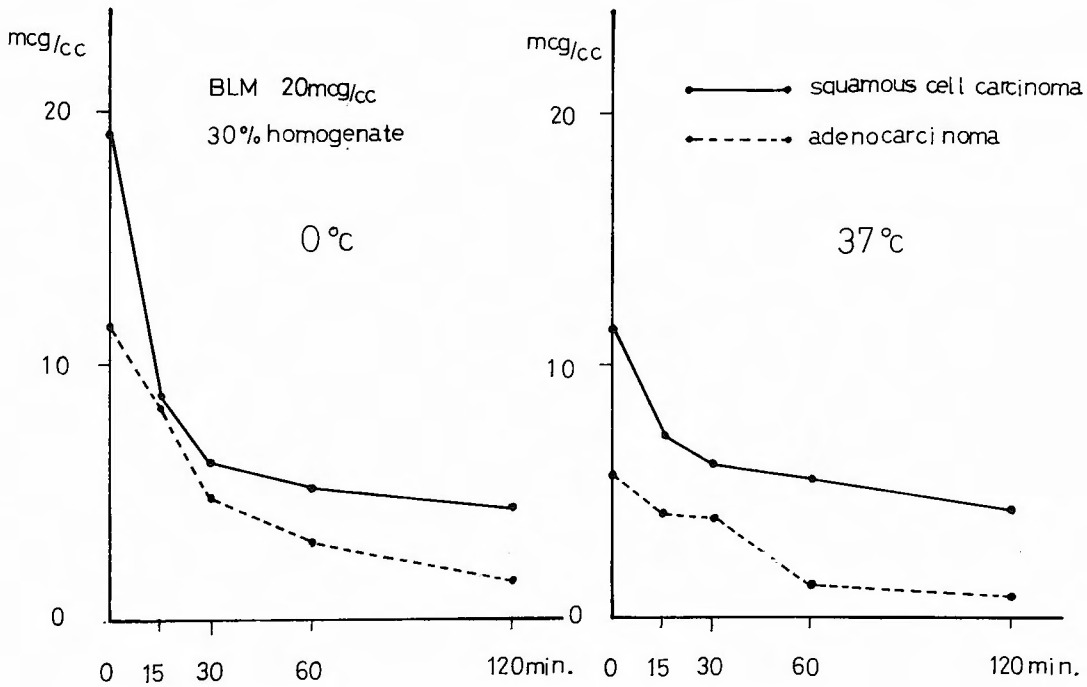

Fig. 8 Inactivation of Bleomycin by tissues of squamous cell carcinoma or adenocarcinoma

Short summary

1) Inactivation of BLM caused by tissue of squamous cell carcinoma was weaker than normal tissue of the esophagus.

2) Inactivation of BLM was thought to be caused by the adsorption and the enzymatic decomposition.

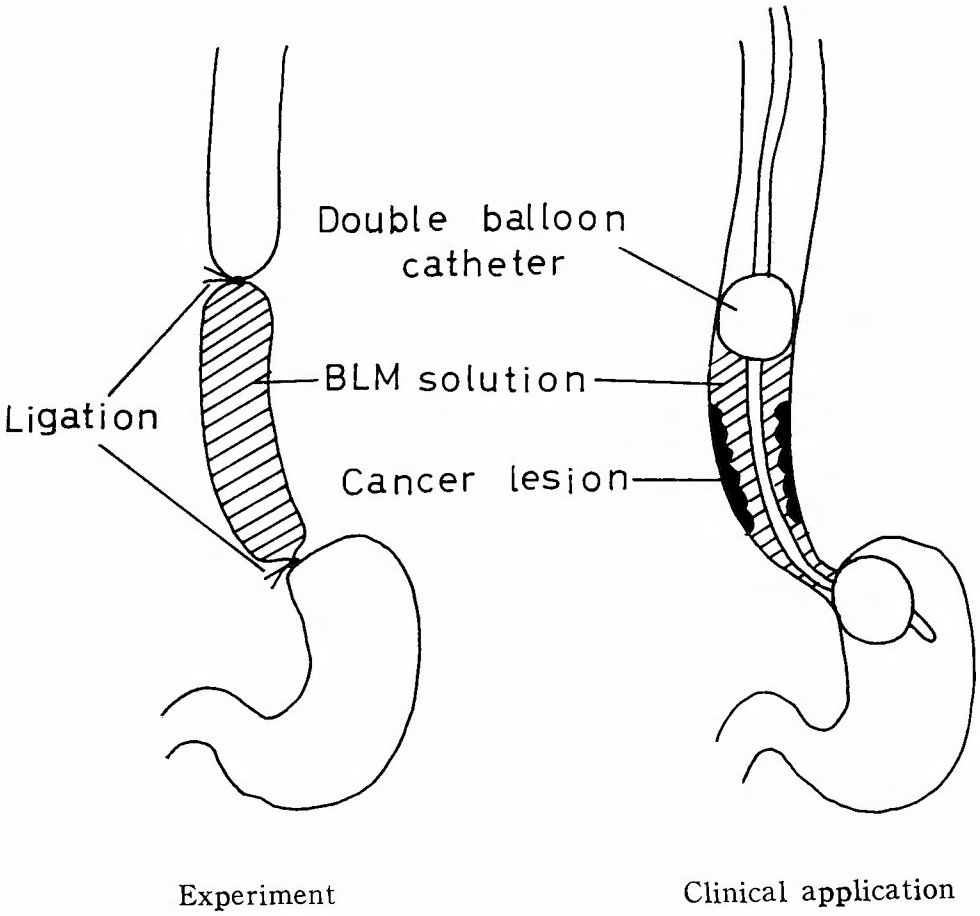

Fig. 9 Model of Bleomycin administration into the lumen of the esophagus

3) BLM has greater effect on squamous cell carcinoma as compared with adenocarcinoma.

Chapter IV. Histological changes of the esophagus in which Bleomycin was administered by intramural injection

Histological changes of the esophageal wall subjected to local injection was investigated, because it is uncertain whether local injection of BLM is safe for clinical cases or not.

Materials and methods

Fifteen mg of BLM in 10 cc of physiologic saline solution was administered by intramural injection into the cervical esophagus of dogs. The tissue of the esophageal wall was investigated macro- and microscopically seven days after the local injection. Gross observation of the tissue was performed on the mucosal and muscular layers of the esophagus, separately. Microscopic observation was performed as follow: The tissue of the esophagus was fixed in 10% formalin solution. The specimen was embedded in paraffine. The paraffine section was prepared. The section was stained by Hematoxylin-Eosin solution.

Results

Each layer of the esophagus which was injected with BLM solution, showed no pathological reaction on gross observation. Inflammatory changes, erosion, ulceration and scar formation were also not observed microscopically (photo. 1). These results may indicate the safety of local injections of BLM in clinical cases.

Chapter V. Histochemical investigation of distribution of Cu-BLM in the esophagus

The injected BLM distributed almost exclusively in the mucous membrane of the esophagus, as shown by the bioassay method. The distribution of BLM in esophageal tissue can be observed from an histochemical point of view. BLM chelates copper in the process of production. Commercial BLM is devoid of the chelated copper in Cu-BLM[55]. Then, in this experiment, Cu-BLM was administered into a mouse. The copper in the tissue of the mouse was stained by the histochemical method. In this way, the location of BLM in the esophageal tissue may be clarified.

Materials and methods

Three mice of dd-strain were used. One hundred mg per kilogram in body weight of Cu-BLM (contained 2.8% of copper) was injected intramuscularly into each mouse. The esophagus of mouse was resected 30, 60 and 120 minutes following the injection, and copper in the tissue was stained by the rubeanhydric acid method by OKAMOTO-UTAMURA[39](Table 15).

Table 15 Histochemical demonstration of copper

A. Material
mouse of dd-strain, 30g in body weight

B. Drug
1 mg/cc of Cu-BLM (content of copper : 2.8%) is intramuscularly injected into mouse

C. Method
Rubeanhydric acid staining
1. Fresh resected tissue is fixed by dehydrated alcohol
2. Paraffin section is prepared
3. Copper in tissue is stained by a solution composed of 5cc of 0.1% dehydrated alcohol solution of rubeanhydric acid and 100cc of 10% sodium acetate solution
4. Washing in water
5. Staining with alum carmine
6. Dehydration and enclosure by barsum

The same doses of Cu-BLM were injected into five other mice, and BLM levels in the esophagus was determined by the band culture method.

Results

The copper in Cu-BLM was stained as dark green colored granules in the epithelium of the esophagus 30 minutes after intramuscular injection. The granules decreased in amount 60 minutes after the injection. Furthermore, 120 minutes later, the copper was not seen (Photos.2, 3 & 4). These results showed a similar tendency to BLM levels in the esophagus on bioassay which decreased gradually in the course of time (Table 16).

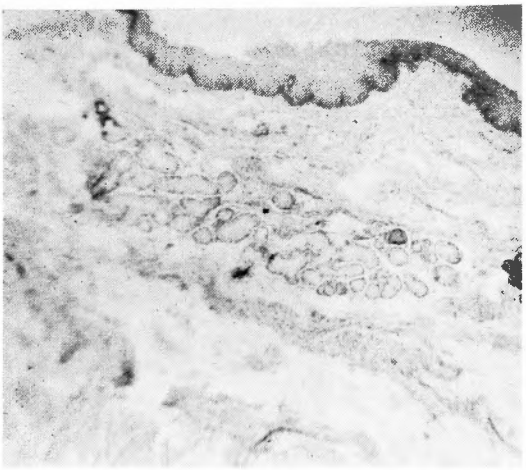

Photo. 1 Seven days after the local injection into the cervical esophagus (×28)

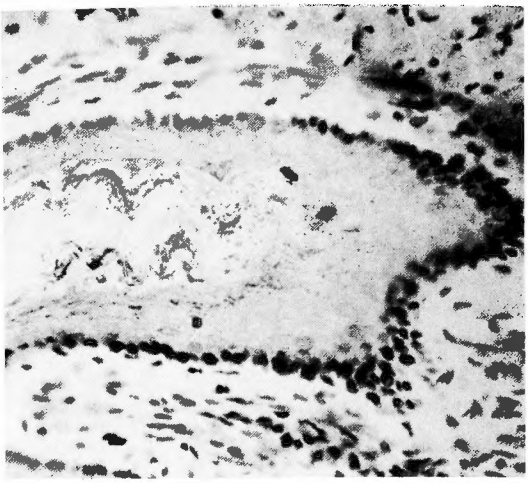

Photo. 2 30 min. after i.v. administration of Cu-BLM (×280)

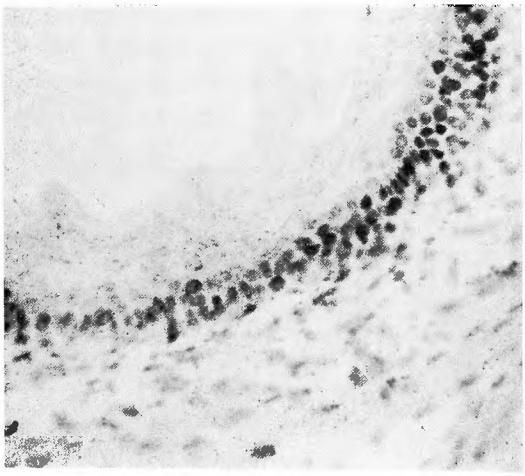

Photo. 3 60 min. after i.v. administration of Cu-BLM

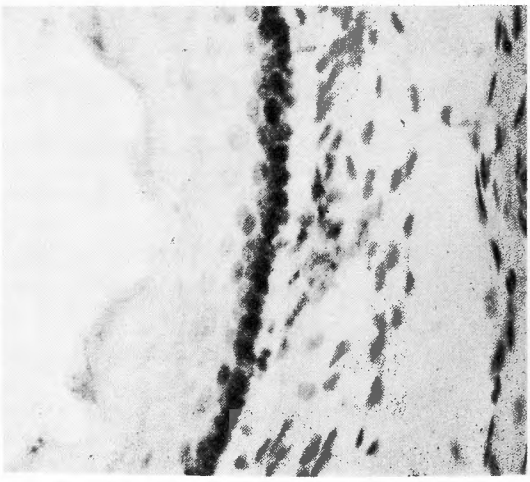

Photo. 4 120 min. after i.v. administration of Cu-BLM

Table 16 Cu-BLM levels in the esophagus
1 mg/cc of Cu-BLM was administered intramuscularly into five mice of dd-strain

Time (min.)	Cu-BLM levels (mcg/g)
30	92.5
60	28.0
120	2.8

Summary

The copper in Cu-BLM was clearly stained after the injection into mice. The majority of injected Cu-BLM distributed in the epithelial layer of squamous cell in the esophagus.

Histochemical findings showed a similar result to that of bioassay. The specific affinity of BLM for the tissue of squamous cell was clarified in this experiment.

Discussion

Both operation and radiotherapy for carcinoma of the esophagus are classified into a local treatment, and their effects may be obtained only with localized lesion in clinical cases. On the other hand, chemotherapy is a general treatment which may have effect on both primary lesions of esophageal cancer and its remote lymphatic dissemination. KARASAWA pointed out that the necessity of chemotherapy for esophageal cancer is as follows[19]: ① When esophageal cancer was found, the cancer lesion had advanced and had occult dissemination as multiple lymphatic or blood vessel invasion. ② Many cases had intramural skip metastases, ③ Both operation and radiotherapy as a local treatment were limited in curability, because esophageal cancer had extensive dissemination into intrathoracic or peritoneal lymph nodes, and ④ Remote lymphatic dissemination and hematogenous metastasis were seen when postoperative recurrence of esophageal cancer had occurred. GREENSTEIN, et al. have concluded the following enzymatic figures of neoplastic cell[8]: ① tumors have qualitatively the same enzymes as normal tissues, ② the enzymatic pattern of a tumer is largely independent of its age, of its growth rate and of the strain of animal in which it is grown, ③ tumors posses a more uniform and less diverse chemical pattern than normal tissue, ④ when a normal tissue becomes neoplastic, many of the specific functional activities markedly decrease or are lost altogether, ⑤ the range of values for the tumors is usually between the extremes of the corresponding values for normal tissues, ⑥ tumors tend to converge, enzymatically, to a common tissue. Therefore, only from the enzymatical viewpoint, so far as the antineoplastic drugs act only upon the enzyme system related to the autonomic atypical multiplication of the neoplasma, its effective antineoplastic action cannot be expected without some severe side-effects on systemic administration[14]. Usually, systemic administration, such as intravenous or intramuscular injection of anticancer agent has been used for cancer of the esophagus[38]. However, such types of administration are not always effective against neoplasm of the esophagus whose arterial supply is relatively poor, as compared with other regions of the digestive tract. The method of administration for esophageal cancer must be suitable for the purpose that high concentration of the drug reaches the lesion.

In 1966, BLM was discovered by UMEZAWA, et. al.. Since ICHIKAWA reported the marked effect of this antitumor drug on squamous cell carcinoma in clinical cases[11][12], this drug was given to many cases of esophageal cancer. Thereafter, BLM has been used as a chemotherapeutic agent for cancer of the esophagus[2][4][18][34]. BLM, a water-soluble basic glycopeptide is produced by streptomyces verticillus and is classified into antitumor antibiotics. BLM consists of A complex ($A_1, A_2, A_3, A_4, A_5, A_6$, and A_2') and B complex ($B_1, B_2, B_3 B_4, B_5$, and B_6). BLM which is used in clinical cases, is in copper-free form mainly composed of A_2. The action of BLM-A_2 inhibits DNA synthesis[16][51][56]. BLM-A_2 inhibits uptake of thymidine and causes strand scissions in DNA molecule. MATSUZAWA, et al. noticed the marked effects of BLM on well differentiated squamous cell carcinoma and lymphogenous tumors, such as Hodgkin's lymphoma or Burkitt's tumor[26]. To clarify this specific effect, the electric charge on cell membrane was determined. Usually, a cell membrane has negative electric charge. The more the cell differentiates, the more the electric charge on cell membrane decreases. Electric

charge on cell membrane in lymphogenous tumor is lower than that in well differentiated type of squamous cell carcinoma. On the other hand, there is a correlation between sensitivity to BLM and electric charge on cell membrane. The sensitivity increases when the charge decreases. The cyto-lethal effect of BLM is attributed to metabolic disturbance which is produced under the influence of electric charge on cell membrane. The specific effect of BLM on squamous cell carcinoma has a close relation to the influence on electric charge on the cell membrane rather than the action upon the enzymatic system of carcinoma[25)26)]. The usual and simple method for the measurement of antitumor antibiotic is bioassay[28)]. This method is based on antibacterial activity of antitumor antibiotic, and it is classified into vertical and horizontal diffusion methods[7)]. The former contains the superposition method, and the latter contain cup, disc and band culture methods[27)54)]. There are thin cylinder plate and band culture methods as microassay of antitumor antibiotic. In this study, the band culture method by OKUBO was used[41)], because it possesses good points of superposition and cup methods. BLM levels of thoracic duct lymph which originated from lymph of the esophagus continued to show high values following intravenous injection of BLM. This result suggests that it has long-acting effects on main tumor and lymphatic dissemination in cancer of the esophagus. High values of BLM were detected in human lymph nodes because most of them belonged to Groupe I among regional lymph nodes of esophageal cancer. The majority of them were possibly invaded by metastases of squamous cell carcinoma. However, BLM levels in lymph nodes in healthy dogs also showed a high level. Therefore, the injected BLM may act effectively on metastatic lesion in the right cervical and contralateral intrathoracic lymph nodes which might not be removed during the operation. Among various side effects of BLM, interstitial pneumonitis and pulmonary fibrosis should be considered before esophageal surgery[2)4)16)56)]. Most cases of esophageal cancer were advanced in years, between 40 and 70. Furthermore, major surgical procedures, such as thoracotomy are performed on the old having unhealthy cardio-pulmonary function. Occurrence of pulmonary complication worsens the results of surgical treatment of the esophageal cancer. On the other hand, it is important to note that BLM in the lung held high levels following its systemic administration. We must be careful about lung complication of BLM at the time of esophageal surgery. When the disturbances of liver, kidney and spleen functions are present, we must be prudent about the use of BLM, because its distribution in these organs has shown the high levels. When an intravenous injection of BLM combined with thoracic duct drainage was administered in dogs[46)], BLM concentrations in the esophagus and lymph nodes were lower than those by intravenous injection alone. This result may be caused by an increase in its clearance from organs due to acceleration of lymphatic flow in cases with lymphatic drainage. The local injection is an excellent method of administration for the prevention of lymph node metastases and pulmonary complication. Local injection of anticancer drug into clinical cases of esophageal cancer were reported by HASHIMOTO and NELSON[10)36)]. However, these reports were not based on experimental studies. The direction of flow in the lymphatic vessels of the upper two-thirds of the thoracic esophagus tends to be upward, whereas in those of the lower one-third it tends to be downward. BLM distribution following intramural injection was influenced by the direction of intramural lymphatic flow[29)30)31)44)]. Accordingly, intramural local injection was effective for the intramural skip lesion in esophageal cancer. When BLM was administered by local injection, its levels in the esophagus was easily altered under the influence of

blood or lymph flow in paraesophageal vessels in association with intramural vessels in the esophagus. This was thought to be due to the development of abnormal lymph flow that the distribution of BLM in remote lymph nodes increased by blockade of the intramural lymphatic flow in the esophagus[13]. It corresponds to SHIRAHA's experimental results studied by RIHSA[48]. The results of our clinical statistical studies also showed that annular cancer infiltration in esophageal wall increased the metastases to remote lymph nodes[15]. In a two-stage operation for cancer of the thoracic esophagus, intramural injection of BLM into the cervical esophageal fistula after subtotal thoracic esophagectomy is an effective method against residual lesion and recurrence in lymph nodes in the neck and in the upper mediastinum. Moreover, BLM distribution was not found in the lung, when it was administered by the local injection. On that occasion, the pulmonary complication seems to occur less frequently, as compared with systemic administration. When BLM is injected into the esophageal wall, we had better repeat it every seven days from the viewpoint of residual time of BLM in the esophagus. Hitherto, the selective intraarterial injections of BLM for carcinoma of the esophagus were administered via the inferior thyroid artery for cancer of the cervical esophagus[23], and via the bronchial artery for cancer of the thoracic esophagus. The author tried to perform it by using the intraarterial injection into the ascending branch of the left gastric artery for cancer of the lower thoracic esophagus. However, this method will sometimes meet with a difficulty, as there are many variations concerning the origin of this artery, such as the celiac or the inferior phrenic arteries, etc. BLM distribution in the esophagus by the intraarterial injection showed low levels. This low level in the esophagus is thought to be due to the interval from the time of one-shot injection[21)49)58]. If BLM is administered by intraarterial infusion, its levels in the lower thoracic esophagus may be higher than those by one-shot or intravenous injections. It was shown that BLM was well absorbed from the esophageal mucous membrane, when it was administered into the lumen of the esophagus. The distribution of BLM was detected in the normal mucous membrane, cancer lesion and regional lymph nodes of the esophagus in cancer patients. BLM levels in cancer lesion or regional lymph nodes by this type of administration was almost identical with those by the intravenous injection. If the cases are adequately selected from among the cancer patients, it can be expected that high concentration will act on main lesion and metastatic lymph nodes when the lumen of the esophagus is obstructed by double balloon catheter at the sites oral and aboral to the lesion. BLM administration per os or by using a catheter will attain its object in cases with complete obstructive lesion of esophageal cancer (Fig. 9). According to KIMURA, et al.,[20] the nature of cytocidal acion of anticancer agents is classified into three groups. The action of drugs in Group IA depends on their concentration, that in Group IB depends both on concentration and time of action, and that in Group II depends on time. BLM and MMC belong to Group IB, while antimetabolites, such as 5-FU, belong to Group II. Judging from the mode of BLM action, such types of local injection, selective intraarterial injection and administration into the lumen of the esophagus, seem to be effective, because BLM may act on the tumor for a long time and in high concentration. Five-FU and MMC levels in the esophagus were lower than BLM levels, because both of them, especially MMC, were inactivated by the esophageal tissue, and further, MMC was administered in a relatively small dose. There are many reports on the administration of BLM alone for cancer of the esophagus[2)4)34]. However, recent reports put emphasis on the combination therapy of BLM

and radiation, because a large dose of BLM is liable to cause pulmonary complication, and makes postoperative care of respiration difficult[45]. Combining radiotherapy with BLM treatment, the dosage of BLM may be decreased[19)50)]. BLM is effective on the well differentiated form of squamous cell carcinoma[2)], while radiotherapy is effective on its undifferentiated form. Recent combination therapies for esophageal cancer are as follows[19)] : total dose of BLM is limited to about 60 to 100 mg, and radiation is given with the dose of 2000 to 3000 rads. BLM inhibits the process of subsequent repair when the strand of DNA is scissored by radiation[53)]. The antitumor effect of BLM is intensified by radiotherapy because the electric charge on cell membrane is lowered by radiation. Such synergic effect on squamous cell carcinoma occurs significantly when BLM is administered during 30 to 60 minutes before or after radiation[26)]. In clinical cases of esophageal cancer, BLM levels in normal esophagus adjacent to the cancer lesion decreased after the preoperative radiotherapy, probably because of the devasation of lymphatics and the fibrosis. This result shows, that supplementary chemotherapy combined with surgical treatment for esophageal cancer should be performed mainly before or during the preoperative radiotherapy from the viewpoint of BLM distribution in cancer lesion and the adjacent normal esophagus and increase of remote lymphatic metastasis or occurrence of postoperative pulmonary complication[1)42)]. The relation between the degree of differentiation of cancer and BLM concentration in cancer lesion is not so clear because the number of investigated cases are very small. BLM distribution in the mucous membrane of the esophagus was detected significantly 30 minutes to 7 days after the administration. This result reveals the affinity of BLM for squamous cell tissue. It is reasonable to presume that BLM accumulates in the esophagus after the local injection, and is excreted little by little gradually into the urine. BLM was not detected in glandular mucosa of the adjacent stomach when it was injected intravenously. This results shows that the affinity of BLM is different in various epithelia. Judging from the results of the inactivation of BLM caused by cancer tissue, it is suggested that BLM should act effectively upon squamous cell carcinoma rather than adenocarcinoma. The adsorption of BLM to the tissue or blood corpuscle occurs at any temperature, while the inactivation by fermentation begins to occur at a constant temperature[6)22)]. BLM was inactivated by both of them. The administration of BLM for cancer of the esophagus has not yet been investigated fundamentally. As shown in the results of the present investigation, supplementary chemotherapy combined with surgical treatment of carcinoma of the esophagus will be applied to clinical cases, and it will make rapid progress more and more in the near future.

Conclusion

For the purpose of improving the surgical curability of esophageal cancer, the features of its lymphatic dissemination were investigated on our clinical cases. The levels of anticancer agents, such as BLM, MMC and 5-FU, in various body fluids and organs of experimental animals and esophageal cancer patients were determined. The results were as follows : 1) Remote lymphatic dissemination became more frequent when the invasion of esophageal cancer reached to the submucosal layer of the esophagus. This tendency was more pronounced when the annular infiltration of the lesion into the esophageal wall became complete and preoperative radiotherapy was performed. 2) Following the intravenous injection of drug, peak level of BLM concentration appeared a little later and persisted for 30 minutes in the

thoracic duct lymph, as compared with that in the peripheral blood. BLM distribution in the esophagus was higher than in other organs. A slight dose of BLM remained in the esophagus, lymph node and lung even 25 to 27 hours after the intramuscular injection of 0.5 to 2 mg/kg of the drug. 3) Relatively high levels of BLM were detected in the esophageal mucous membrane, but not in the muscular layer following the intravenous injection of the drug in dogs or clinical cases. Slight concentration of 5-FU was distributed in the esophageal muscular layer of dogs, while MMC levels were not detected in the esophagus of dogs and clinical cases. Following intravenous injection of BLM in esophageal cancer patients, the drug levels in the lesion were higher than those in the adjacent normal esophagus and decreased after the preoperative radiotherapy of betatron. Copper ion in the esophagus of mice of dd-strain was histochemically detected by rubeanhydric acid method by OKAMOTO-UTAMURA following the intramuscular injection of Cu-BLM into them. The results were completely identical with those obtained by the bioassay method. 4) BLM was administered into dogs by local injection, selective intraarterial injection and intravenous injection. BLM levels in the esophagus and intrathoracic regional lymph nodes increased following local injection of the drug into the esophageal wall, intravenous injection and selective intraarterial injection into the ascending branch of the left gastric artery, in the order mentioned. BLM levels in the lung were lowered following intravenous injection, selective intraarterial injection and local injection, in the order mentioned. The thoracic duct drainage combined with local injection into the esophageal wall decreased the drug levels, while ligation of the thoracic and the right lymphatic ducts or the azygos and the intercostal veins caused the prolongation of intraesophageal drug level. When BLM was injected into the wall of the lower thoracic esophagus, the distribution of the drug was observed in the sites oral and aboral to the injected portion. BLM was detected in the cervical and intrathoracic lymph nodes, such as 105 and 107, following intramural injection into the cervical esophageal fistula. No pathological changes were found in the injected portion 7 days after intramural injection of 15 mg of BLM solution into the cervical esophagus in dogs. 5) When BLM was administered into the lumen of the esophagus in dogs and cancer patients, the drug levels in the normal esophageal mucous membrane, cancer lesion and regional lymph nodes were observed at the levels as high as those following intravenous injection. 6) Inactivation of BLM by cancer tissue of squamous cell type was weaker than those by the normal esophageal epithelium and adenocarcinoma. BLM was inactivated by the adsorption and the enzymatic decomposition of various tissues.

From these results it can be said that it is necessary to combine surgical treatment with supplementary chemotherapy and radiotherapy, so as to improve the operative curability of esophageal cancer. On such occasion, supplementary chemotherapy should be carried out before or during the preoperative radiotherapy. Furthermore, it is emphasized that BLM should be administered by such methods, as local injection, selective intraarterial injection and administration into the lumen of the esophagus, but not by systemic administration, lest lung complication, etc. might occur.

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和文抄録

食道癌手術に併用する制癌剤療法に関する研究

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食道癌手術の根治性を向上するために，食道癌47例についてリンパ行性転移型式を再検討するとともに，イヌ，マウスおよび食道癌臨床例について帯培養法(大久保)を用いて BLM, MMC および 5-FU の体液中または臓器内濃度を測定した。一方 BLM の扁平上皮癌組織に対する親和性の本態を解明するために不活性化試験の成績および組織化学的所見から検討を加え，次のような成績を得た.

1) 食道癌の癌浸潤が食道粘膜下層に達すると遠隔部リンパ節転移が多発し，その傾向は術前照射の施行や癌浸潤の壁全周波及によって助長された.

2) BLM を静注すると，胸管リンパ中の濃度は末梢血のそれより遅れて極値に達し，以後は高値を持続した。臓器別にみると，食道に最高濃度で分布し，リンパ節や肺にも高値をもって分布した.

3) BLM の静注では，イヌ，ヒトともに正常食道粘膜層のみに分布し，筋層には分布しなかった．5-FU はイヌの食道筋層のみに僅かに分布し，MMC はイヌおよびヒトの食道には検出できなかった．食道癌臨床例では癌腫部には正常食道部より高濃度に分布し，Betatron 照射群では非照射群に比べて低濃度で分布し，脈管系の荒廃がその原因と考えられた.

4) dd 系マウスに含銅ブレオマイシンを投与し，ルベアン水素酸法（岡本・宇多村）で銅を検出した結果，含銅ブレオは食道の重層扁平上皮層に特異的に分布し，経時的にその分布量は減少し，同時に測定した臓器内濃度の消長とも一致した.

5) BLM をイヌに食道壁内局注，左胃動脈上行枝への選択的動注および静注の3方法で投与すると，食道および胸腔内所属リンパ節における濃度は局注＞静注＞選択的動注の順となり，肺内濃度は静注＞選択的動注＞局注の順となった．局注による食道内濃度は胸管ドレナージで低値となり，右リンパ総管と胸管の結紮，あるいは奇静脈と肋間静脈の結紮によって高値となった．BLM を胸部下部食道壁内に局注すると，壁内リンパ流にのって頭側および尾側の食道にもよく移行し，一方壁内リンパ流を絹糸による結紮や全層連続縫合でブロックすると食道内停滞をきたし，遠隔部リンパ節における分布が増加した。また頚部食道褒粘膜下に BLM を局注すると，頚部食道の所属リンパ節や105, 107などの胸腔内リンパ節に分布し，肺内には分布しなかった点から，この方法は分割手術時の頚部や上部縦隔のリンパ節再発に対処しうる方法と考えられた.

6) BLM 15 mg 水溶液の頚部食道内局注後7日には BLM は注入部食道内になお残留していたが，同部にはなんらの組織学的変化も認めなかった.

7) イヌ，食道癌臨床例のいずれにおいても食道内腔内投与によって正常食道粘膜層，癌腫部および所属リンパ節などに静注に比して勝るとも劣らない分布を示し，末梢血や胸管リンパにも移行した.

8) BLM の扁平上皮癌組織による不活性化は正常食道上皮および腺癌のそれらより弱く，しかも BLM の不活性化は組織の吸着と酵素反応によることが判明した.

以上の成績から，食道癌手術の根治性を拡大するためには適当な制癌剤療法を併用することが必要であり，しかも手術に術前照射および BLM 療法を併用するさいには，肺合併症の発生に注意するとともに，照射前および照射中に投与することが望ましいこと，さらに BLM 投与法としては全身投与のみでは不充分であり，食道壁内局注，選択的動注または食道内腔内投与などを適切に選択することが必要であることを強調した。